

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
14 December 2000 (14.12.2000)

PCT

(10) International Publication Number
WO 00/74485 A1

(51) International Patent Classification⁷: **A01N 43/04**,
A61K 31/70, **C12Q 1/68**, **C12P 19/34**, **C07H 21/02**,
21/04, **21/00**

(21) International Application Number: **PCT/US00/15243**

(22) International Filing Date: **2 June 2000 (02.06.2000)**

(25) Filing Language: **English**

(26) Publication Language: **English**

(30) Priority Data:
60/137,470 **4 June 1999 (04.06.1999)** **US**

(71) Applicant (for all designated States except US): **THE BOARD OF REGENTS THE UNIVERSITY OF TEXAS [US/US]**; 201 West 7th Street, Austin, TX 78701 (US).

(72) Inventors; and

(75) Inventors/Applicants (for US only): **ROY, Arun, K.** [—/US]; 8415 Fredericksburg Road, Unit #1006, San

Antonio, TX 78229 (US). **LAVROVSKY, Yan [RU/US]**; 7701 Wurzbach Tower, #1507, San Antonio, TX 78229 (US). **TYAGI, Rakesh, K. [IN/US]**; 4900 Medical Drive, Apartment #624, San Antonio, TX 78229 (US). **SONG, Chung, S. [US/US]**; 15023 Northern Dancer, San Antonio, TX 78248 (US). **CHATTERJEE, Bandana [US/US]**; 8415 Fredericksburg Road, #201, San Antonio, TX 78229 (US).

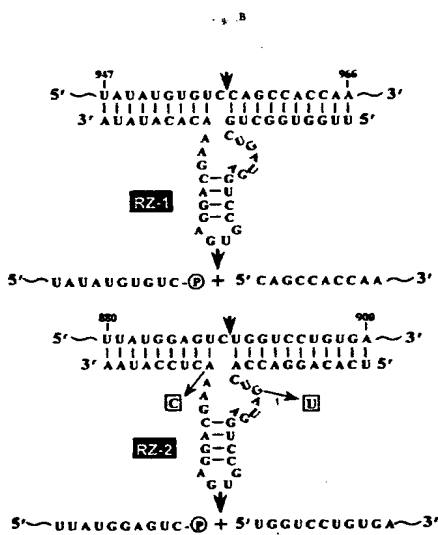
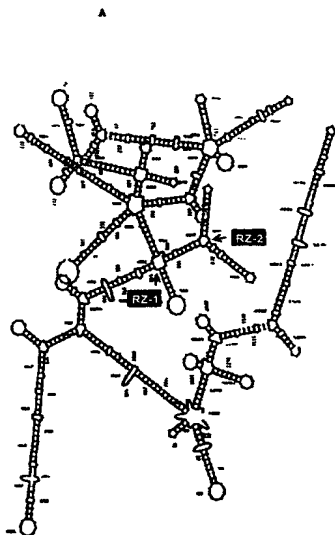
(74) Agent: **MAYFIELD, Denise, L.**; Locke Liddell & Sapp, LLP, Suite 2200, 2200 Ross Avenue, Dallas, TX 75201-6776 (US).

(81) Designated States (national): **AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW.**

(84) Designated States (regional): **ARIPO** patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), **Eurasian** patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), **European**

[Continued on next page]

(54) Title: **ESTROGEN RECEPTOR SITE-SPECIFIC RIBOZYMES AND USES THEREOF FOR ESTROGEN DEPENDENT TUMORS**



(57) Abstract: Highly specific hammerhead ribozymes are provided that human target estrogen receptor mRNA. These ribozymes, designated RZ1 through RZ7 provide predictable mRNA cleavage products. Methods for inhibiting estrogen-dependent tumor growth, such as that characteristic of breast cancer, are also provided employing these ribozymes. One or both of the ribozymes may be used together or separately with equal efficiency. The ribozymes possess a sequence region with a catalytic core that provide the attributed catalytic activity to these ribozymes.

WO 00/74485 A1



patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

— Before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments.

Published:

— With international search report.

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

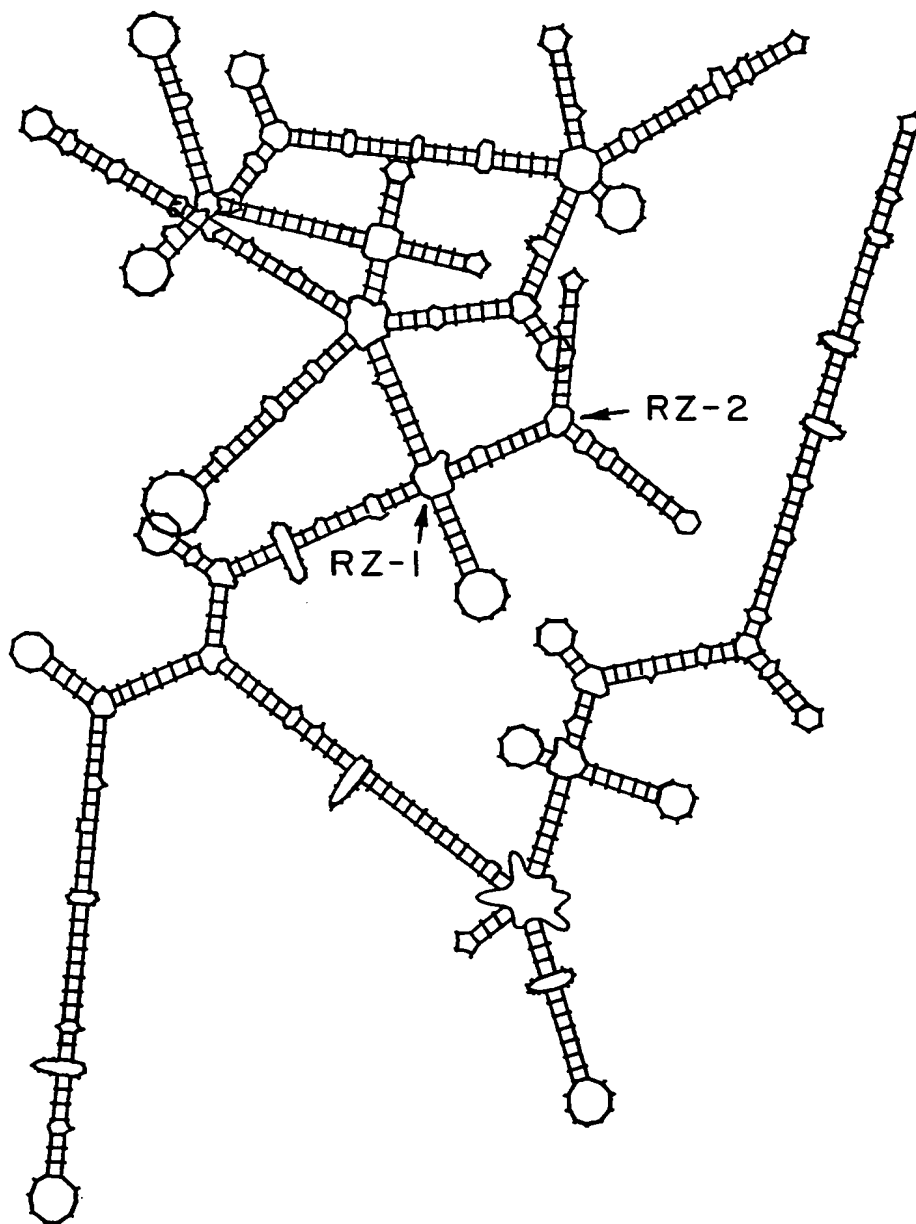


FIG. 1A

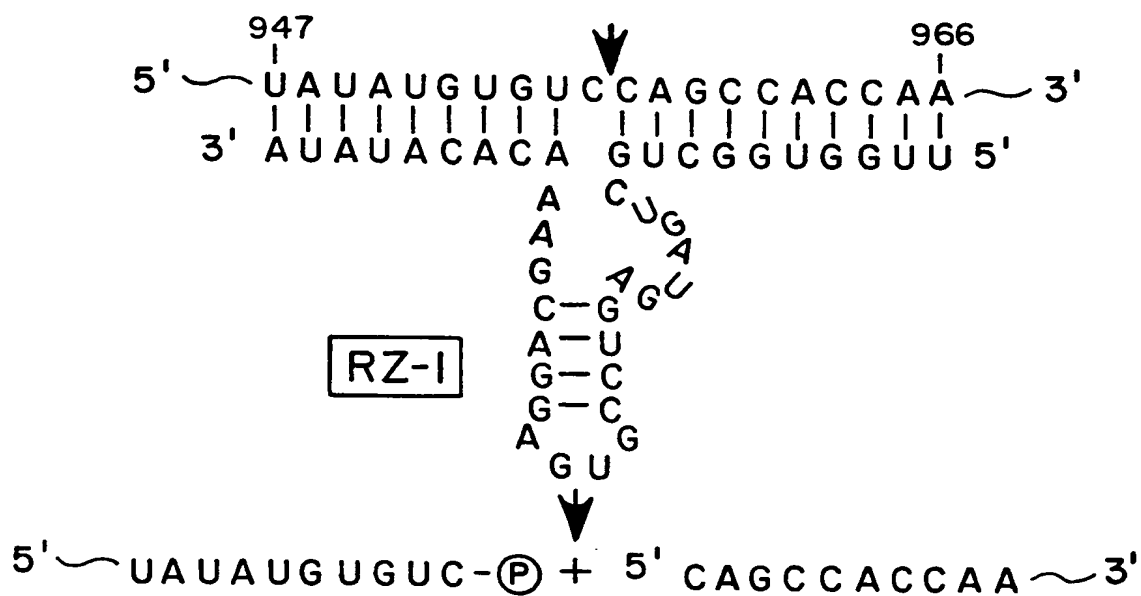


FIG. 1B

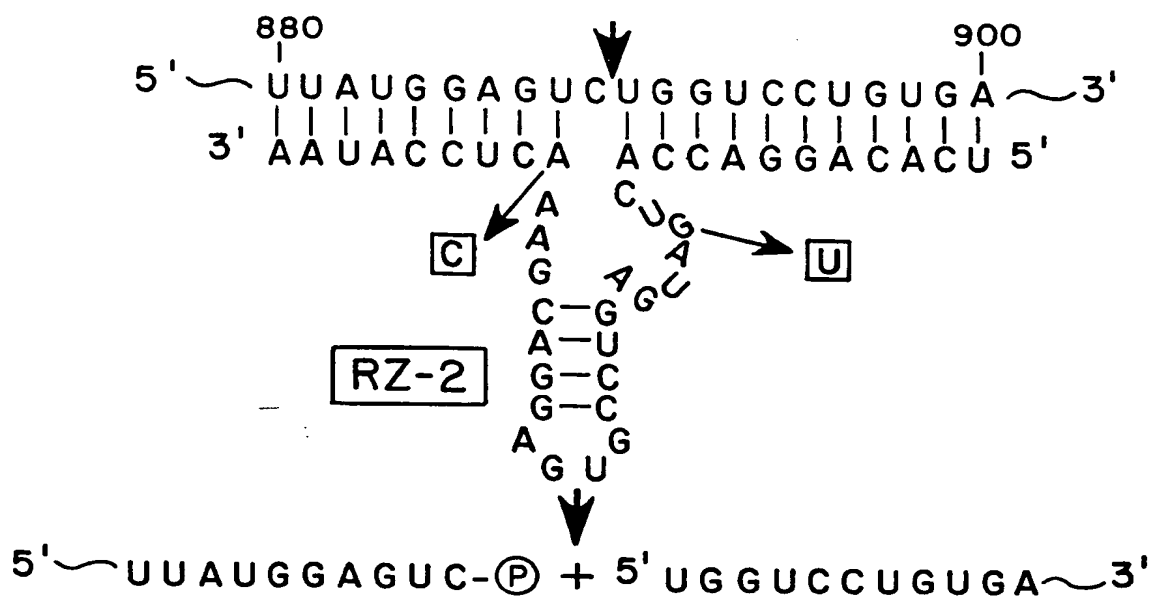
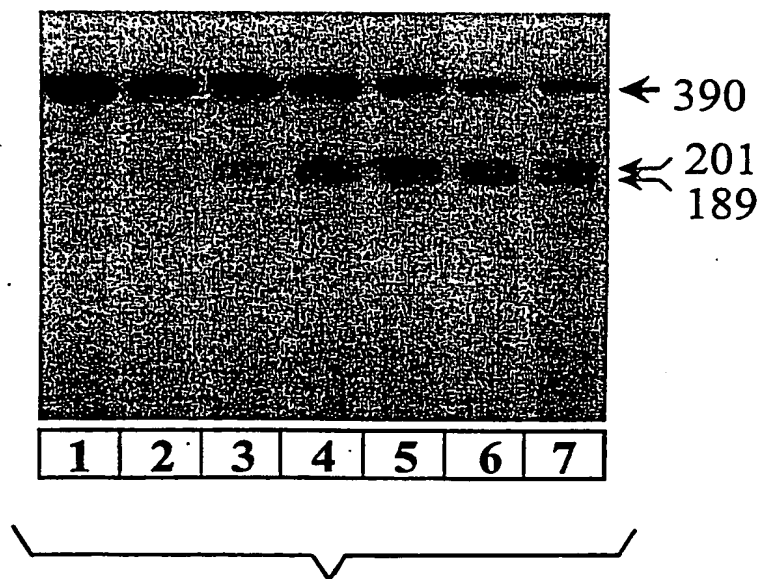
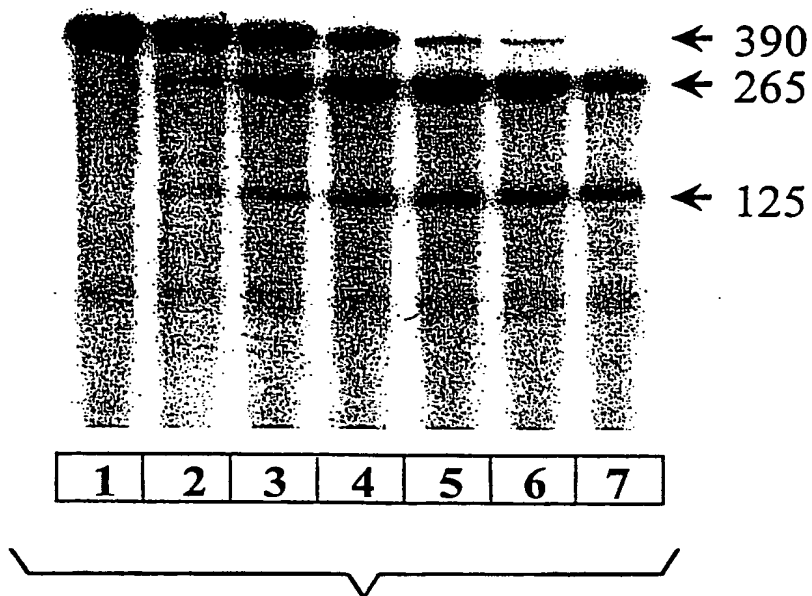


FIG. 1C



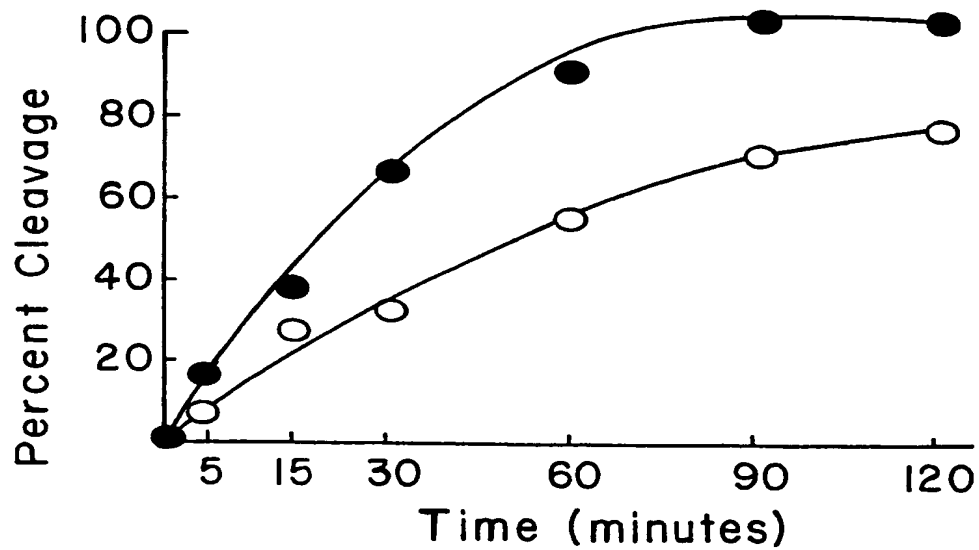


FIG. 2C

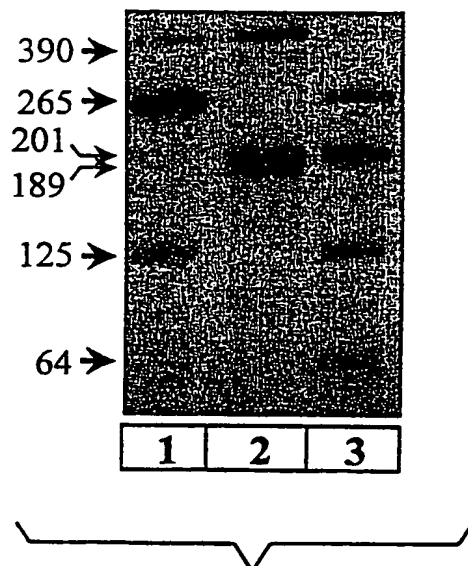


FIG. 2D

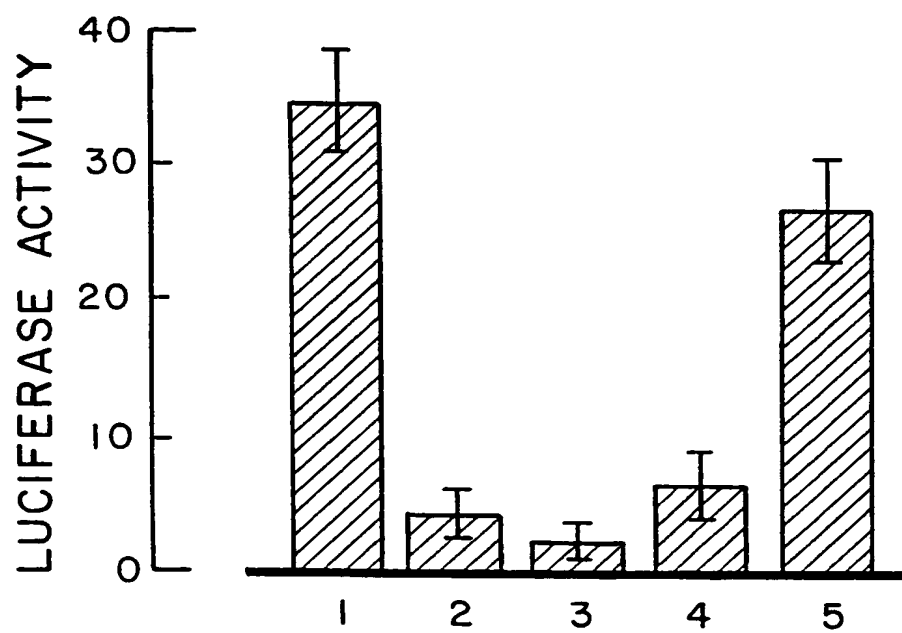


FIG. 3A

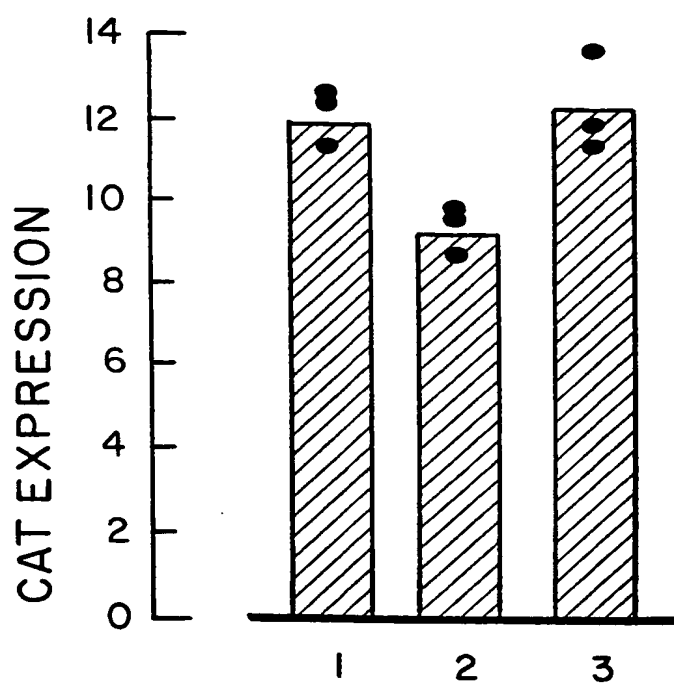
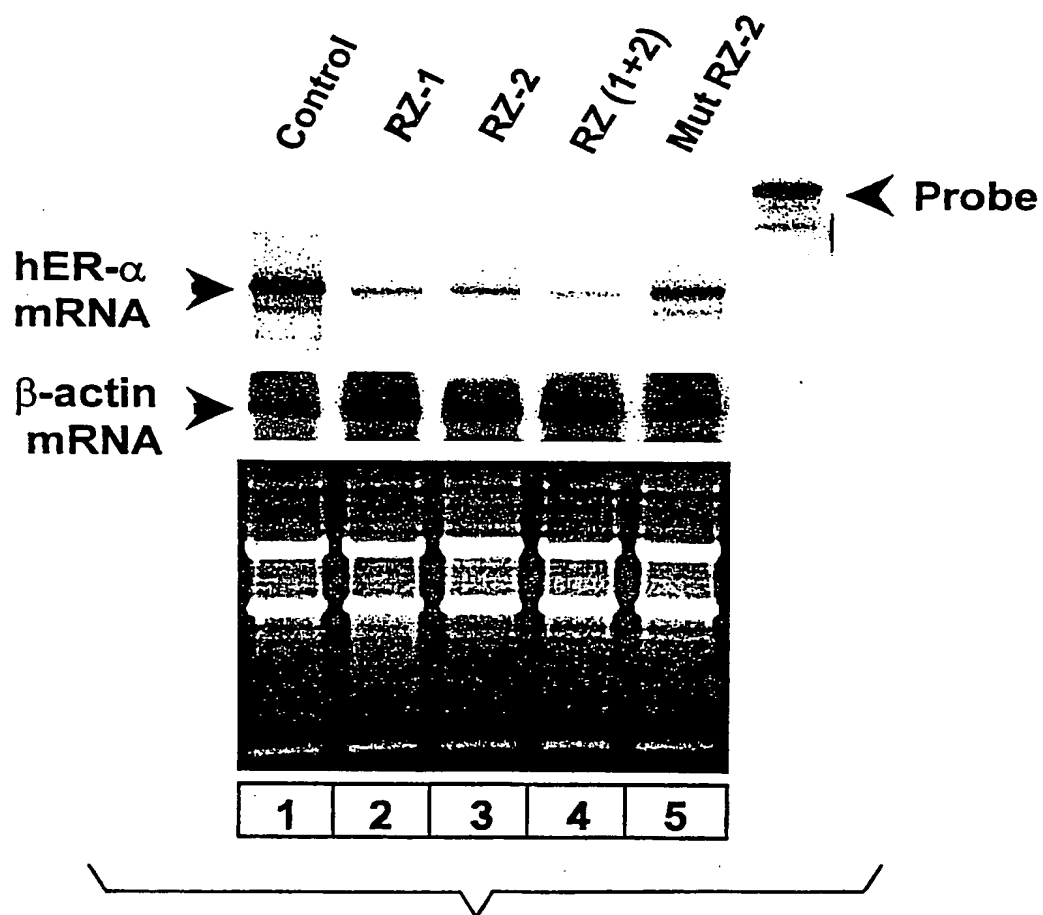


FIG. 3B



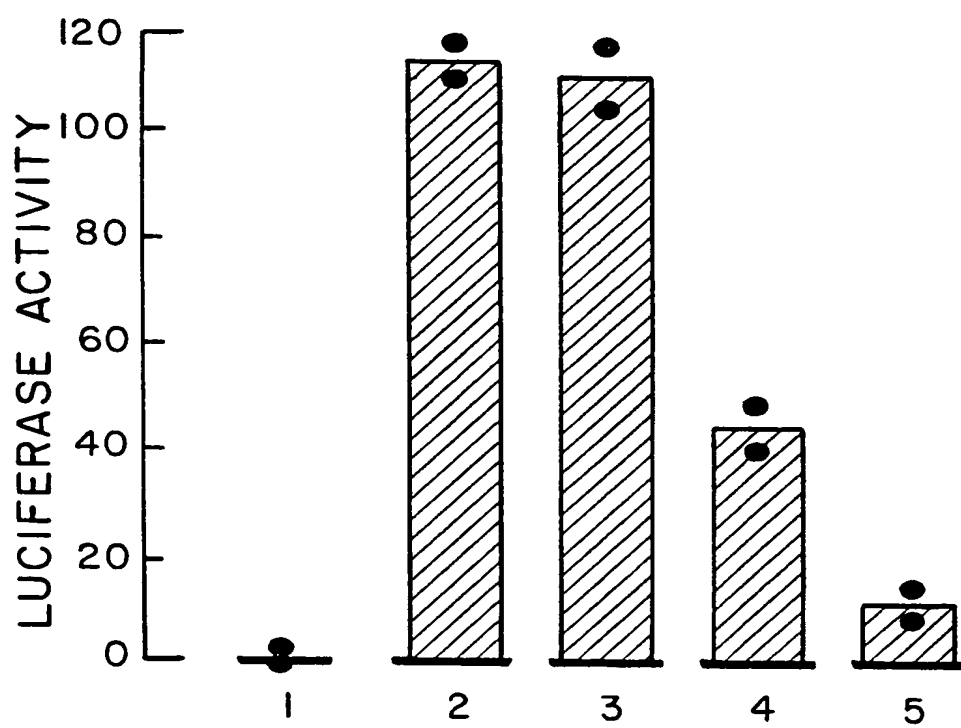
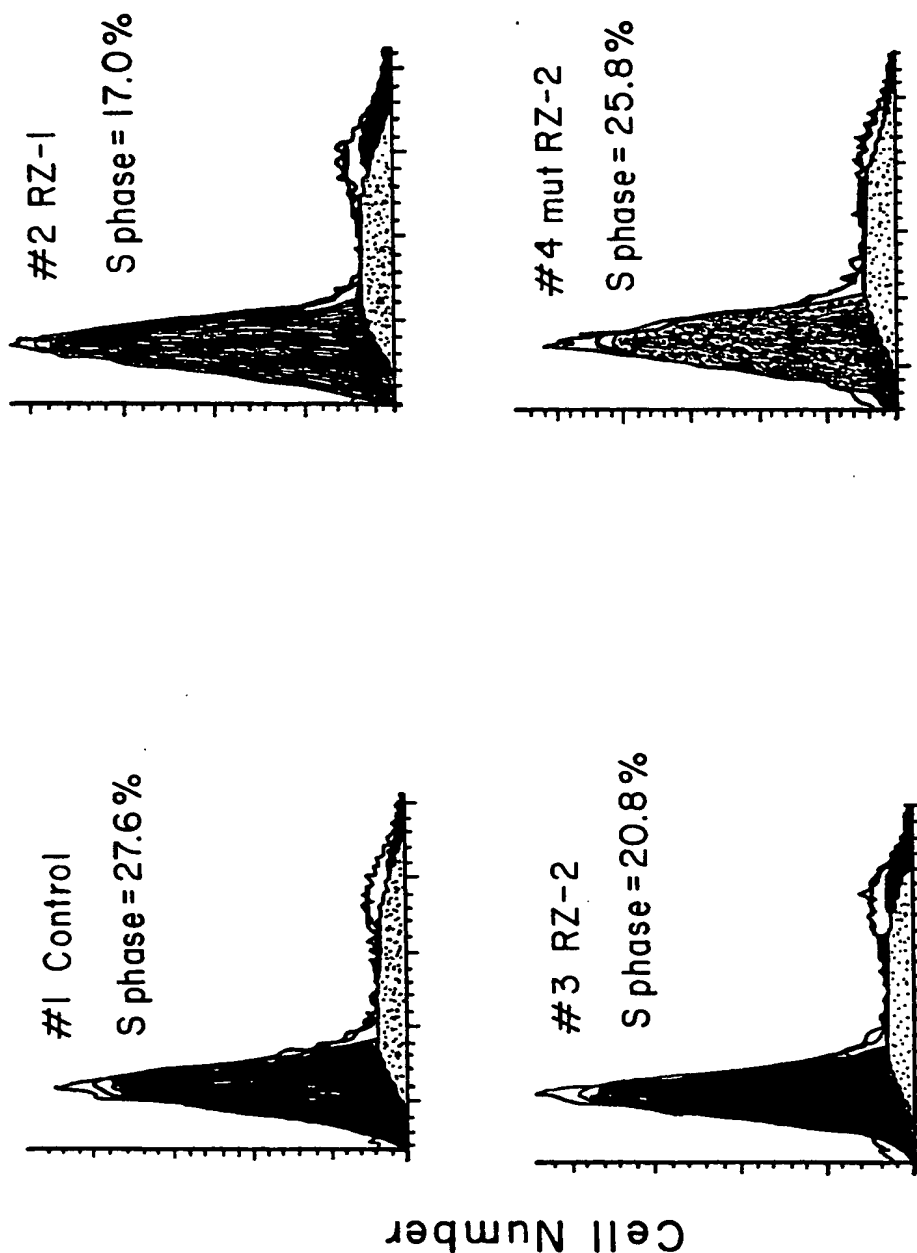


FIG. 5



Relative PI Fluorescence

FIG. 6

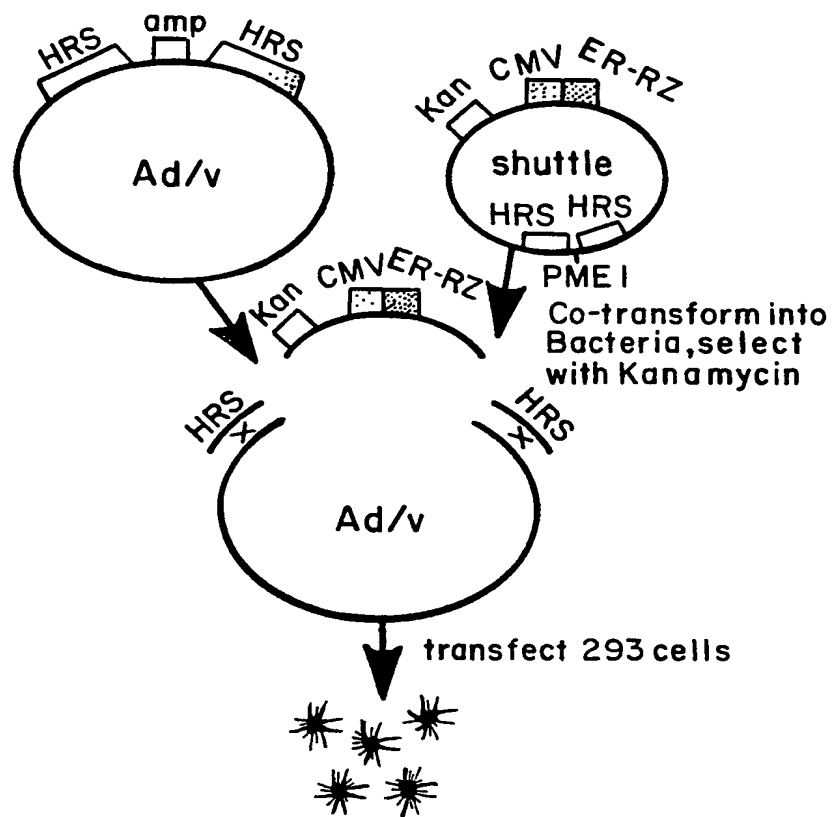
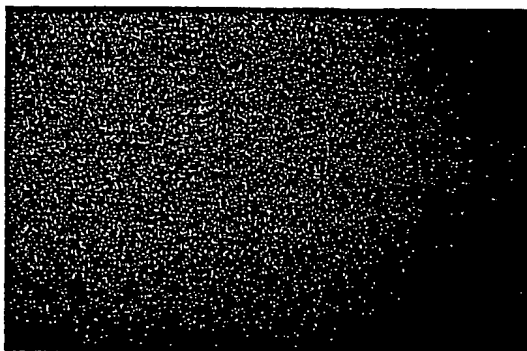
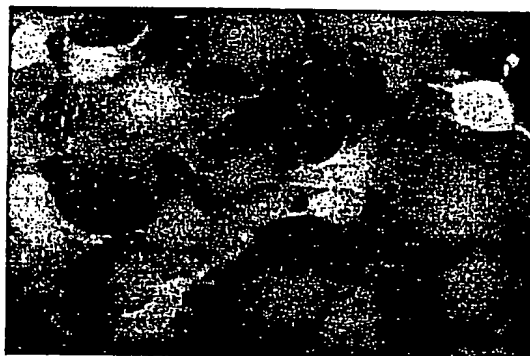


FIG. 7



Control cells

FIG. 8A



Ad/v-Rz 2 infected cells

FIG. 8B




Ad/v-Rz 2-M infected cells

FIG. 8C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/15243

A. CLASSIFICATION OF SUBJECT MATTER IPC(7) :Please See Extra Sheet. US CL :Please See Extra Sheet. According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) U.S. : 514/44; 435/6, 91.1, 91.31, 91.5, 455, 366, 375; 536/ 23.1, 24.5, 25.3 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Dialog, West		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	KLEFSTROM et al. C-Myc induces cellular susceptibility to the cytotoxic action of TNF-alpha. The EMBO Journal. 1994, Vol. 13, No. 22, pp. 5442-5450, entire text, especially section entitled :Inhibition of TNF and cMyc-induced apoptosis by Bcl2 and MnSOD", pages 5444-5445.	1-22
A	TURLEY et al. Vitamin E succinate induces Fas-mediated apoptosis in estrogen receptor-negative human breast cancer cells. Cancer Research. 1997, Vol. 57, pp 881-890, see especially last paragraph of intro on page 881 and discussion on pages 887-888.	1-22
<input checked="" type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
* *A* *E* *L* *O* *P*	Special categories of cited documents: document defining the general state of the art which is not considered to be of particular relevance earlier document published on or after the international filing date document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified) document referring to an oral disclosure, use, exhibition or other means document published prior to the international filing date but later than the priority date claimed	*T* *X* *Y* *A* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art document member of the same patent family
Date of the actual completion of the international search 11 SEPTEMBER 2000		Date of mailing of the international search report 04 OCT 2000
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230		Authorized officer  Jane Lara Telephone No. (703) 306-5820

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/15243

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 99/54459 A2 (RIBOZYME PHARMACEUTICALS, INC.) 28 October 1999 (28.10.99), page 23, line 29-page 27, line 17 and figure 17).	1, 6
Y	MILNER et al. Selecting effective antisense reagents on combinatorial oligonucleotide arrays. Nature Biotech., 1997, Vol. 15, pp 537-541, see entire text.	1-22
Y	JAMES, W. Towards gene-inhibition therapy: a review of progress and prospects in the field of antiviral antisense nucleic acids ribozymes. Antiviral Chem. and Chemotherapy, 1991, Vol. 2, No. 4, pp 191-214, see especially pages 197-198.	1-22

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/15243

A. CLASSIFICATION OF SUBJECT MATTER:

IPC (7):

A01N 43/04; A61K 31/70; C12Q 1/68; C12P 19/34; C07H 21/02, 21/04, 21/00

A. CLASSIFICATION OF SUBJECT MATTER:

US CL :

514/44; 435/6, 91.1, 91.31, 91.5, 455, 366, 375; 536/ 23.1, 24.5, 25.3